Impact of Multiwalled Carbon Nanotubes for the Vegetative Growth and Yield Attribute of Wheat (*Triticum aestivum* L.)

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INTRODUCTION

Nanotechnology

The term "nano technology" was defined by Tokyo Science University Professor Norio Taniguchi in a 1974 paper (Taniguchi, 1974) as follows: " 'Nano-technology' mainly consist of the processing of separation, consolidation, and deformation of materials by one atom or by one molecule."

In 1980s the basic idea of this definition was explored in much more depth by Dr. K. Eric Drexler, who promoted the technological significance of nano scale phenomena and devices through speeches and the books Engines of Creation: The coming Era of Nanotechnology (1986) and Nanosystems: Molecular Machinery, Manufacturing, and Computation (Drexler, 1991), and so the term acquired its current sense.

The nano technology can be exploited in the value chain of the entire agriculture production system (Subramanian and Tarafdar, 2011). Nanotechnology is emerging as the sixth revolutionary technology in present after the industrial revolution of mid 1700s, nuclear energy revolution of the 1940s, the green revolution of 1960s, information technology revolution of 1980s and biotechnology revolution of 1990s. The nanotechnology aided applications have the potential to change agriculture production by allowing better management and conservation of inputs of plant.

Nanotechnology is the creation and utilization of materials devices and systems through the control of the properties and structure of the matter at the nonmetric scale. Targeted research and development to understand, manipulate and measure of the materials with atomic, molecular and super molecules dimensions is called nanotechnology. Nanotechnology is a wide field and has found application in most of the fields of science. Nanotechnology has the potential to revolutionize the agriculture with new tools to enhancing the absorption of nutrients by the plants.

Nano usually refers to a size scale between 1nm and 100nm. Nano particles can serve as 'magic bullets' containing herbicides, chemicals or genes which target particular plant parts to release their content. Nano capsules can enable effective penetration of herbicides through cuticles and tissues, allowing slow and constant release of the active substance (Perea – de Lungue and Rubiales, 2009).

Convergence of technology with biology at the nano level is called nanobiotechnology. Nano-biotechnology is a highly interdisciplinary field of research and is based on the co operative work of physicists, chemists, biologists, doctors, engineers (Prasanna, 2007).

The credit for the term nanobiotechnolgy goes to Lynn.W. Jelinski, a biophysicist at Cornell University, USA. Nano particles interact the living cells at molecular level and nano agriculture involves the employment of nano particles in agriculture. These particles impart some beneficial effects to the crop. The use of nano particles for growth of plants and the control of plant diseases is a recent practice.

Carbon Nanotubes:

Carbon nano tubes (CNTs) are tubular of Buckminster fullerene was discovered by Iijima in 1991. They are straight segments of carbon hexagonal units. CNTs have superior electrical, mechanical and thermal properties. CNTs are devided as single walled carbon nano tubes (SWCNTs) and multi walled carbon nano tubes (MWCNTs).SWCNTs formed by a single graphene sheet and MWCNTs are formed by several graphene sheets wrapped around the tube core(Sinnott, 2002). The range of the diameter of the carbon nano tubes is of a few nanometers and their length is upto several micrometers. Interaction between plants and CNTs needs to be investigated from the cellular to the organismic level to understand its multifaceted complexity. This will pave the way to develop the technology of 'nanoagriculture' a new area of biotechnology that holds promise for growth acceleration and higher productivity of crop plants (Srinivasan and Saraswathi 2010).

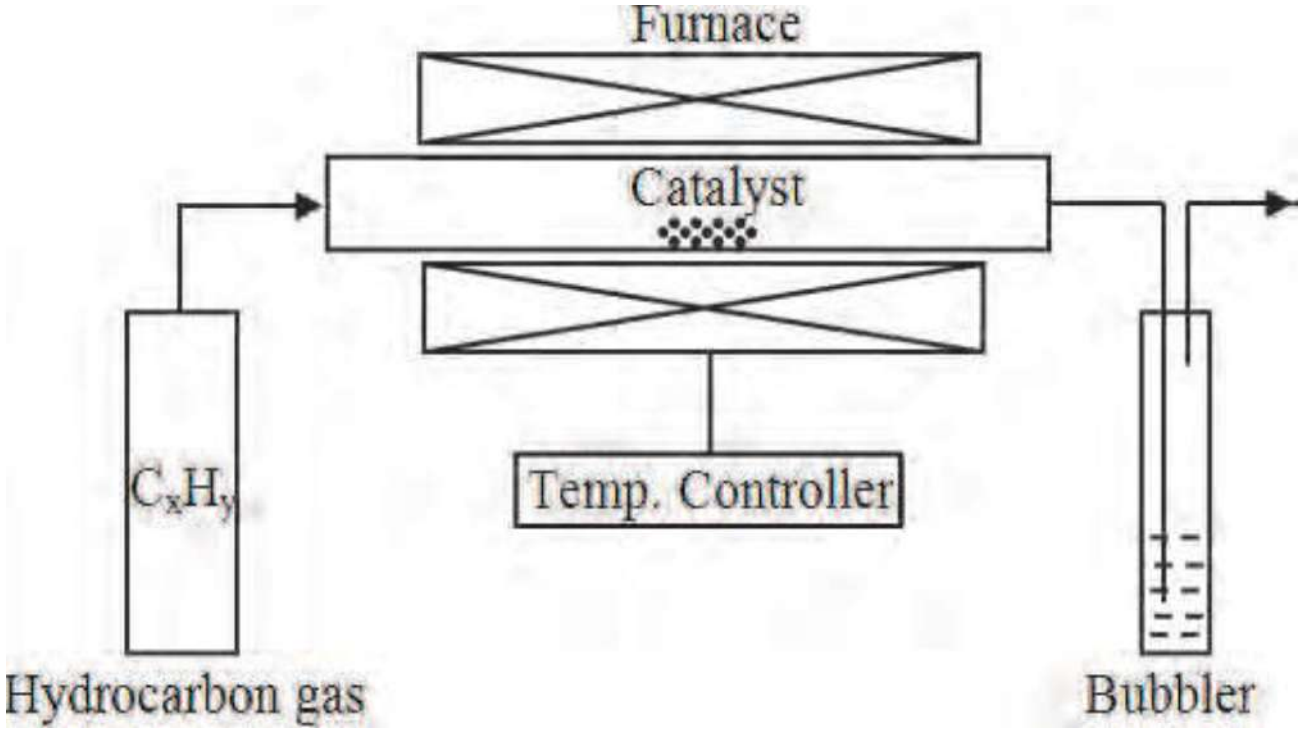
Many researchers have reported the dramatic effects of multi walled carbon nano tubes (MWCNTs) on seed germination and plant growth. MWCNTs have been shown to penetrate the seed coat and stimulate the growth of tomato (Khodakovskaya*et al*., 2009; Villagarcia*et al*., 2012) and mustard seeds (Mondal*et al*., 2011). Water soluble MWCNTs have been shown to exhibit similiar dramatic improvement of the growth of gram plant (Tripathi*et al*., 2011).

Unique features of carbon nanotubes :

- CNT is 100 times strongerand six times lighter than stainless steel.
- CNT is as hard as diamond and its thermal capacity is twice that of pure diamond.
- CNT's current-carrying capacity is 1000 times higher than that of copper.
- CNT is thermally stable up to 4000K.
- CNT can be metallic or semiconducting, depending on their diameter and chirality.

Principle of Synthesis of Carbon Nanotubes:

According to the principle of Green Chemistry, the feedstock of any industrial process must be renewable, rather than depleting a natural resource. The process must be designed to achieve maximum incorporation of the constituent atoms into the final product. Hence it is the time's prime demand to explore regenerative material for CNT synthesis with high efficiency. The Chemical Vapour Deposition method(CVD) involving catalyst assisted thermal decomposition of hydrocarbons, is the most popular method of producing CNTs and it is truly a low- cost and scalable technique for mass production of carbon nanotubes.(Cassel., *et al*., 1999; Couteau, *et al*.,2003). Success has been achieved in growing gram quantities of CNTs from camphor, a botanical product. Camphor is simply extracted from the latex of *Cinnamomum camphora* tree belonging to the family lauracea (Mukul Kumar and Yoshinori Ando, 2008).



Schematic diagram of a CVD setup in its simplest form.

Description of test crop:

Wheat (*Triticum aestivum* L.)

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Poales
Family	Poaceae
Subfamily	Pooideae
Tribe	Triticeae
Genus	*Triticum*
Species	*aestivum*

Wheat is grown all over the world; wheat covers more of the earth's surface than any other cereal crop. However, although it takes more land space than other cereals, it is only the third-largest cereal crop, behind maize and rice. The domestication of grains and the development of agricultural lifestyles led to significant changes in people's lives, encouraging permanent settlements, the development of civilization and trade.

Wheat plantings for rabi season has completed at over 227.82 lakh hectares in 2012. Wheat acreage is up for rabi season in M.P, Rajsthan, Assam, Bihar, Chhattisgarh and Jharkhand, while it is lower in Maharastra,Uttarakhand, Gujrat, West Bengal, Karnataka and Haryana.

Wheat cultivars can be classified by planting season, hardness of the grain, and color. Winter wheat are winter hardy, so they are planted in the fall. In the spring they resume maturation and are harvested early in the summer. Spring wheat are planted in the spring and harvested late in the summer. Spring wheat yields are significantly lower than winter wheat yields, but it offers a very high quality for bread making. Soft wheat and white wheat usually bring higher prices because they are easier to mill and don't require bleaching.

The wheat looks much like grass when it first sprouts, then continues to grow until it 'heads out.' In the Punjab region of India and Pakistan, as well as North China, irrigation has been a major contributor to increased grain output. More widely over the last 40 years, a massive

increase in fertilizer use together with the increased availability of semi-dwarf varieties in developing countries, has greatly increased yields per hectare. In developing countries, use of fertilizer increased 25-fold in this period. However, farming systems rely on much more than fertilizer and breeding to improve productivity.

Origin: Soft Wheat (Bread wheat) – Hindukush mountainous regions adjoining to India and Afghanistan.

The optimum temperature range for growth is between 7^0C to 21^0C. The rainfall requirement is 750 to 1600 mm/year. Hot and humid climate is harmful because it encourages the infestation of diseases like rust, root rot etc. In early growth stage, it requires low temperature and dew formation which increases tillering. Wheat crop is grown in different types of soils ranging from desert soil to heavy clay soil. Well drained, fertile clay loam soils having moderate water holding capacity are ideal for irrigated wheat.

Cereal grains store energy in the form of starch. The amount of starch contained in a wheat grain may vary between 60% and 75% of the total dry weight of the grain. Starch occur in seed in the form of granules. 100 gm of wheat grains produces 330 Kcal energy and contains 13.10 gm water, 78.18 gm carbohydrate, 12.61 gm protein, 1.54 gm fat, and 1.57 gm minerals.

Economic Importance:

1. Wheat is the staple food of North Indian people.
2. Wheat grains are grounded into flour and consumed in the form of chapatee i.e. 80- 85%.
3. Soft wheat is used for making chapatee, bread, cake, biscuits, pastry and other bakery products.
4. Hard wheat is used for manufacturing rawa, suji and sewaya.
5. In areas where rice is a staple food grain, wheat is eaten in the form of puri and uppumav.
6. It is also used for making flakes and sweet meats like kheer, shira, etc.
7. Wheat grain is used for preparing starch.
8. Wheat straw is used as fooder, padding material and mulching material.

Origin of the problem:

Agriculture field is facing many problems such as stagnation of yield in crops, low nutrients use efficiency, declining soil organic matter, multinutrient efficiencies, climate change, shrinking arable land and water availability and shortage of labor besides exodus of peoples from farming. To address these problems there is a need to explore one of the frontier technologies such as Nanotechnology to precisely detect and deliver the optimal quantity of nutrients and pesticides that promote productivity while ensuring environmental safety and higher use efficiency.

Carbon nano tubes amongst all the nano materials have a major role because it has multi dimensional properties like mechanical, electrical, thermal and chemical. Most of the research of CNTs in the biosciences have focused on their influence on animal cells (Donaldson *et al*. 2006 Martinelli *et al*. 2012). Very little attention has been paid to the effect of CNTs on the plant cells (specially for crops) and the way that might influence the physiology and the development of the plant. Khodakovskaya *et al*., (2009) have reported that they planted tomato seeds in a soil that contained carbon nanotubes; these CNTs penetrate into the hard coat of germinating tomato seeds and exerted growth enhancing effect. They envisaged that the enhanced growth was due to increased water uptake caused by penetration of CNT. CNTs can be used for boosting up the early germination process as well as disease resistance by providing desired molecules and reducing the lifecycle of the crop.

Now a days it is very common practice followed by most of the farmers for applying fertilizers by broadcasting method along with sowing and before sowing, causing wastage of most of fertilizers due to either volatilization or leaching which is not become available to the plants for proper germination and seedling growth. As plants become dried just after sowing due to very little moisture available or increase in atmospheric temperature resulting most of the seeds do not germinate or dried just after germination. In order to overcome these constraints it is expected that by optimization of fertilizers and greater moisture uptake with the application of carbon nanotubes these wastage of nutrients can be minimized ultimately resulting in increase in crop yield. Keeping these views in mind present work entitled “Impact of Multiwalled Carbon Nanotubes for the Vegetative Growth and Yield Attribute of Wheat (*Triticum aestivum* L.) as been proposed for this study.

REVIEW OF LITERATURE

Wierzbicka *etal*., (1993) observed that plant and plant cells showed high tendencies to accumulate CNTs making plants as an important link in the pathway by which CNTs enter the food chain and biological cycles.

Andreas and Angew (2002) observed that diameter is an important dimension in determining the properties and applications of tubular carbon nanostructures. Small single walled carbon nanotubes (SWNT) diameter is strongly correlated to synthesis technique, the diameter inducing higher strain energies, mixing of σ and α bonds and electron orbital rehybridization. These bond structure modifications induce fundamental alterations to the electronic, optical, mechanical, elastic and thermal properties of SWCNTs.

Huang *et al*., (2004) Hydrophilic CNTs have increased dispersivity and are able to provide better surface contact with biological adsorbates than the hydrophobic CNTs.

Samaj *et al*., (2004) demonstrated that SWCNTs of length less than 500 nm labeled with fluorescein isothiocyanate (FITC) penetrate the cell wall of the living plants by endocytosis. FITC alone is not easily taken up by the plants. Which means that both of them jointly facilitate the absorption/penetration of nanomaterials.

Biswas and Wu (2005) observed that a wide range of nanoparticles such as metal oxide nanoparticles (ZnO, TiO_2, Al_2O_3, etc.) fullerenes, carbon nano tubes, quantum dots, etc. an increasing range of applications for different purposes make their way easily in the environment . Their potential adverse effects on the environment and human health are being subjected to intense debate (Monica and Cremonini 2009).

Wong *et al*., (2005); Zhu *et al*., (2007) investigated that carbon nanotubes in contrast to other nanoparticles due to their ability to penetrate mammalian and bacterial cells.

Holt *etal*., (2006) demonstrated that the characteristic properties dependent on nanotube diameter are complemented by physical size exclusion and capillary behavior relevant to

environmental and agricultural systems. The narrow inner diameter of nanotubes has found application in novel molding, separation and size exclusion processes.

(Wang *et al*., 2007a, Wang *et al*., 2007b) The equilibrium time of adsorption for acidified CNTs decreases because oxygenous functional groups form chemical complex with metal species and aggregated on the ends and Heavy Metals Removal by Carbon Nanotubes 1015 the defects sites on the acidified CNTs.

Lu and Su (2007) reported that thermally treated CNTs are more efficient for the removal of natural organic matter than the raw CNTs. Thermally treated (heating in oven or annealing) CNTs have larger surface area than the raw CNTs.

Harrison and Atala (2007);Panyam and Labhasetwar (2003); Zanello *et al*.,(2006) have been reported beneficial effects of nano particles in different fields like drug delivery, biosensing etc.

Torney *et al*., (2007) demonstrated that CNTs can assist the delivery of biological molecules into plant cells. The uptake, translocation and accumulation of nanoparticle depend on the species of plant and the size, type, chemical composition, fictionalization and stability of nanoparticles.

Canas *et al*., (2008) demonstrate that nanotubes have the ability to cross plant cell walls and membranes which will alter essential biochemical processes necessary for plant growth and survival.

Liu *etal*., (2008) demonstrated that the capability of single walled carbon nano tubes to penetrate the cell wall and membrane of tobacco cells.

Gonzales-Melendi *et al*., (2008) reported nanoparticle as smart treatment delivery systems in plants. Compared to plant cell walls and membranes, the penetration of nanoparticles into seeds is expected to be difficult due to the significantly thick seed coat covering the whole seed.

Liu *et al*., (2009) pointed out that SWCNTs can serve as effective nano transporters to deliver DNA and small dye molecules into intact plant cells and other biological samples in a soil environment.

Liu *et al.*, (2009) investigated the capability of single-walled carbon nanotubes (SWNTs) to penetrate the cell wall and cell membrane of intact plant cells. Confocal fluorescence images revealed the cellular uptake of both SWNT/fluorescein isothiocyanate and SWNT/DNA conjugates, demonstrating that SWNTs also hold great promise as nanotransporters for walled plant cells.

Khodakovskaya *et al.*, (2009) demonstrated that the exposure of carbon nanotubes to seeds of valuable crops, such as tomatoes, can increase the germination percentage and support and enhance the growth of seedlings. Further these findings could result in significant developments of improved plants for the area of energy, by taking advantage of the enhancement in the biomass of the plants when they are exposed to nanosized materials and fertilizers.

Mondal *et al.*, (2011) reported that the beneficial effect of multiwalled carbon nanotubes having dimeter of ~ 30 nm on *Brassica juncea* seeds. Encouraging results using low concentration of oxidized multiwalled carbon nanotubes treated seeds as compared to non oxidized as well as high concentration OMWCNTs treated seeds.

Tripathi *et al.*, (2011) stated that water soluble carbon nanotubes (wsCNTs) show enhancement of the growth rate of common gram (*Cicer arietinum*) plants. Treating plants with up to 6.0 μg mL^{-1} of wsCNT shows an increased growth rate in every part of the plant including the roots, shoots and also in branching.

Smimova *et al.*, (2011) observed the effect of the industrial material Taunit, containing multiwalled carbon nanotubes on plants and testing of its ability to penetrate in to plant cells and tissues. Taunit stimulate the growth of roots and stems and cause an increase in peroxidase activity in Onobrychisarenaria seedlings. Peroxidase activity increases with decreasing concentration of Taunit from 1000 to 100 mg/l.

Miralles *et al.*, (2012) have been reported that the effect of industrial grade multiwalled CNTs (75 wt % CNTs) and their impurities on alfalfa and wheat. The germination of both species were tolerant of up to 2560 mg/l CNTs and root elongation was enhanced in alfalfa and wheat seedlings exposed to CNTs. Catalyst impurities also enhanced root elongation in alfalfa seedling as well as wheat germination.

Serag *et al*., (2012) reported that cup stacked cellulose impregnated CNTs to penetrate the cell wall and transport intracellularly through cellulose- induced nano holes.

Wang *et al*., (2012)described that MWCNTs increased the fresh weight and root length of wheat seedling but had no effect on seed germination and shoot length.

Begum *et al*., (2012) reported MWCNTs reduced the root fresh weights of rice and cucumber seedling while the root length varied in a nonorderly manner with MWCNT concentration. The germination rate of maize and rye grass decreased with 2000 mg/l of MWCNT but their root lengths increased **(Lin and Xing 2007)**

Jackson *etal.*, (2013) reported that CNT do not cross biological barriers readily. When internalized, only a minimal fraction of CNT translocate into organism body compartments. The reported CNT toxicity depends on exposure conditions, model organism, CNT-type, dispersion state and concentration. In the ecotoxicological tests, the aquatic organisms were generally found to be more sensitive than terrestrial organisms. Invertebrates were more sensitive than vertebrates. Single-walled CNT were found to be more toxic than double-/multi-walled CNT.

Liang,*et al*., (2013) reported that the application of carbon nanoparticles promoted tobacco plant growth and increased nutrition absorption and accumulation amount, thereby increasing fertilizer efficiency and improving tobacco quality.

Tiwari *et al*., (2013) observed that multiwalled CNTs were seen to enhance the germinative growth of maize seedling at low concentration but depress it at higher concentrations. MWCNTs affect mineral nutrient supply to the seedling through the action of the mutually opposing forces of inflow with water and retention in the medium by the ion-CNT transient dipole interaction.

Husen and Siddiqi (2014) observed both the functionalized and non functionalized carbon nanomaterials influence fruit and crop production in edible plants and vegetables. The fullerene, C60 and carbon nanotubes have been shown to increase the water retaining capacity, biomass and fruit yield in plants. In certain cases, non functionalized multi-wall carbon nanotubes are toxic to both plants and animals but the toxicity can be drastically reduced if they are functionalized.

Srivastava and Rao (2014) investigated about the beneficial effects of functionalized multiwalled carbon nano tubes on wheat, peanut and garlic. Low dose MWCNTs have seen to be beneficial, improving water absorption, found to accelerate the process of germination by shortening the germination time and higher biomass production.

OBJECTIVES

(i) To synthesize multiwalled carbon nanotubes.

(ii) To characterize multiwalled carbon nanotubes.

(iii) To study the role of multiwalled carbon nanotubes (MWCNTs) on the germination efficiency of Wheat (*Triticum aestivum* L.).

(iv) To study the effect of MWCNTs on the seedling growth of Wheat (*Triticum aestivum* L.) under control condition.

(v) To estimate the yield parameters of MWCNTs treated Wheat (*Triticum aestivum* L.).

METHODOLOGY

Synthesis of Multiwalled Carbon Nanotubes:

There are two significant methods for synthesizing CNTs.

(i). **Electrodeposition technique** –Carbon nanotubes will be grown on Si substrate using acetonitrile (1% v/v) and water as electrolyte at an applied d.c. potential ~20 V. This technique besides being scalable and cost-competitive would allow coating on irregular surfaces. (Pal et al., 2004)

(ii). **Chemical vapour deposition (CVD)** - Chemical vapor deposition (CVD), incorporating catalyst-assisted thermal decomposition of hydrocarbons, is the most popular method of producing CNTs; and it is truly a low-cost and scalable technique for mass production of CNTs (Cassell et al., 1999).

Carbon nanotubes will be synthesized by using a suitable method from the above.

Characterization of Multi Walled Carbon Nanotubes:

Characterization of MWCNTs will be made by adopting following techniques:-

(i**) Field emission scanning electron microscopy (FESEM)** - SEM would be used to visualize the morphology of CNTs prepared.

(ii**) FTIR –** FTIR would be used to determine the nature of bond in CNT, which in turn will again confirm CNT formation.

(iii) **X-ray diffraction –** XRD would be used to confirm CNT formation.

(iv) **Raman spectroscopy** – It is used to analyze the crystallinity and the diameter distribution of carbon nanotubes.

Experimentation:

Seed collection:

Good quality Foundation/certified seeds of Wheat (*Triticum aestivum* L.) will be procured from seed stores and the R.S.S. Agricultural farm Dayalbagh Agra for present study.

Pretreatment:

Seeds will be initially surface sterilized with 0.1% $Hgcl_2$ for 1 minute. One lot of seeds will be immersed in distilled water and another lot in MWCNTs solution for 6 hours. Seeds will be stirred frequently and excess solution will be decanted. After completion of the treatment the

seeds will separately surface dried with blotting paper and dried back to original weight under sun. Other seeds will not soaked in any solution but sun dried along with treated seeds. After pretreatment the seeds will be stored in normal laboratory condition in perforated paper bags and used after 15 days for experimental purpose.

Preparation of nano particle solution:

The multiwalled carbon nanotubes will be suspended directly in double distilled water by sonication in an ultrasonic bath. MWCNTs solution will be prepared at different concentrations.

Bioassay experimentation :

Sterilization the glass wares before conducting an experiment in the autoclave at 120 ^{o}C, 15 lb/cm^{3} pressure for 15 min. and Wheat (*Triticum aestivum* L.) seeds will be also sterilized by $HgCl_2$ before starting of experimentation.

Germination Assay:

Before starting germination all seeds will be immersed in 10% Sodium hypochlorite for surface sterilization. To analyze percentage of seed germination 50 seed samples will be transferred to petridishes containing filter paper moistened with 10 ml of distilled water (control) or nanoparticle solution (treatment). Germination data will be recorded at every 24 h interval by following rules for International Seed Testing Association, ISTA (1976). Seeds will be considered to be completely germinated when the radicle attained a length of 1 mm and plumule has just unfolded.

Field experimentation: Field experimentation will be done in microplots arranged in Randomize Block Design with three replicates. All the recommended package of practices will be followed uniformly in all the plots except different treatment made by MWCNTs and untreated sown plots will be considered as control.

Growth parameters: No. of plant stand/unit area, shoot length, root length, No.of Tillers, height of the plant, fresh weight, dry weight,

Yield parameters: Number of seeds/spike, grain size, seeds/plant,100 seeds weight .

Observation and analysis: The frequent observations of the experimental plots will be made at different stages during the experimentation and obtained data will be analyzed statistically.

SIGNIFICANCE:

Currently, research is underway to develop nano-composites to supply all the required essential nutrients in suitable proportion through smart delivery system. However its use in agriculture is very negligible. Preliminary results suggest that balanced fertilization may be achieved through nanotechnology. New nutrient delivery systems that exploit the porous nanoscale parts of plants could reduce nitrogen loss by increasing plant uptake. Fertilizers encapsulated in nanoparticles will increase the uptake of nutrients.

In the next generation of nano fertilizers, the release of the nutrients can be triggered by an environmental condition or simply released at desired specific time. CNTs have been incorporated in various polymers such as polyvinyl alcohol, polypropylene and polyamide. Carbon nanotubes are allotropes of carbon whose nanostructure is cylindrical in shape.

These nanotubes have many applications in the fields of nanotechnology, electronics, and architecture. It is used as thermal conductors. These CNTs penetrate into the hard coat of germinating seeds and exerted growth enhancing effect.

The enhanced growth was due to increased water uptake caused by penetration of CNT. This could be a boon for using CNT as vehicle to deliver desired molecules into the seeds during germination that can protect them from the diseases, ultimately to increase the crop productivity. Thus the adoption of CNTs may be an innovative approach in the field of crop production technology.

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